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# Validating the Novel Method of Measuring Cortisol Levels in Cetacean Skin by use of an ACTH Challenge in Bottlenose Dolphins

Thea Bechshoft
Aarhus University
Bioscience Roskilde Frederiksborgvej 399
P.O. Box 358
4000 Roskilde, Denmark
Email: thbe@dmu.dk

Award Number: N000141310771 http://www.au.dk

#### LONG-TERM GOALS

Our research group, under PI Thea Bechshøft, has recently shown that it is possible to extract and measure cortisol in cetacean skin (Bechshoft et al. Submitted). The goal of the current project is to validate this novel method by measuring baseline cortisol levels in cetacean skin and demonstrating the correlation between an acute stress exposure and the ensuing skin cortisol response.

#### **OBJECTIVES**

At the conclusion of the project period, we expect to have provided a greater understanding of a) the relationship in cetaceans between blood cortisol levels and skin cortisol levels, b) the period of time it takes before an acute stress response is measurable in the cetacean skin matrix, and c) cetacean baseline cortisol levels in skin, as well as inter- and intra-individual fluctuations.

#### **APPROACH**

The project is executed under PI Dr. Thea Bechshoft, in collaboration with Dr. Dorian Houser (National Marine Mammal Foundation, USA), Dr. Bjarne Styrishave (Copenhagen University, Denmark), and Dr. Andrew J. Wright (George Mason University, USA and Aarhus University, Denmark).

The project will validate the recently developed skin cortisol analysis method (Bechshoft et al. Submitted) using skin samples collected in connection with an already scheduled stress test, known as an ACTH (adrenocorticotrophic hormone) challenge, in a group of bottlenose dolphins (*Tursiops truncatus*). The dolphins will be sampled as part of an ongoing study of stress hormones conducted by Dr. Dorian Houser in collaboration with the U.S. Navy Marine Mammal Program (MMP). The ACTH challenge process will involve a single, intramuscular injection of ACTH gel followed by sampling from various matrices post-injection. For the skin cortisol validation project, non-invasive skin samples will be collected from a total of five animals at three points in time: pre-ACTH challenge, at time of ACTH challenge, and post-ACTH challenge.

The results of the ACTH challenge will determine the link between the level of cortisol in the blood and the skin of bottlenose dolphins. The results will also establish the delay between a spike in blood cortisol levels and any corresponding spike in skin cortisol levels, thus helping to validate the use of skin cortisol to assess chronic stress levels in bottlenose dolphins and other cetaceans. All skin samples will be analyzed for cortisol levels at the University of Copenhagen, Denmark (Bechshoft et al. Submitted).

#### WORK COMPLETED

Bottlenose dolphin ACTH pilot studies are proceeding as scheduled. Post-ACTH challenge skin collection is expected to be finished by mid-2014 at the latest, after which the samples will be shipped to Denmark for analysis.

# **RESULTS**

Bottlenose dolphin ACTH pilot studies are currently ongoing. Post-ACTH challenge skin collection is expected to be finished by mid-2014 at the latest, after which chemical lab analysis and statistical data analysis will ensue.

# **IMPACT/APPLICATIONS**

A validated method for assessing cortisol in non-invasively collected cetacean skin samples will bring new possibilities for stress assessment in cetaceans, opening up a new avenue of research in physiological response studies following exposure to stressors. The current study will provide the validation of this novel method (Bechshoft et al. Submitted) by measuring basal level cortisol in cetacean skin and demonstrating the correlation between an acute stress exposure and the ensuing skin cortisol response.

# **REFERENCES**

Bechshoft TØ, Wright A, Weisser JJ, Teilmann J, Dietz R, Hansen M, Björklund E & Styrishave B. Developing a novel stress research tool for free-ranging cetaceans: recovering cortisol from harbor porpoise skin. Submitted to *Proceedings of the Royal Society, B*.

# **PUBLICATIONS**

Bechshøft TØ, Wright AJ, Teilmann J, Dietz R, Hansen M, Weisser JJ & Styrishave B. Developing a novel stress research tool for free-ranging cetaceans: recovering cortisol from harbour porpoise skin. Abstract accepted for presentation in connection with the 20th Biennial Conference on the Biology of Marine Mammals Dunedin, New Zealand, 9-13 December 2013.

Bechshoft TØ, Wright A, Weisser JJ, Teilmann J, Dietz R, Hansen M, Björklund E & Styrishave B. Developing a novel stress research tool for free-ranging cetaceans: recovering cortisol from harbor porpoise skin. Submitted to *Proceedings of the Royal Society, B*.